DOI:10.31557/APJCB.2026.11.3.729

RESEARCH ARTICLE

# Enhanced Cytotoxicity of Niosomal Copper Schiff-Base Complexes in MCF-7 Cells: Substituent Effects and Delivery Efficiency

**Hosniyeh Forouzani[1,2], Ehsan Zare Mehrjardi[3], Hadi Kargar[4], Houriyeh Forouzani[5], Mahmood Dehghani Ashkezari[1,2]**

[1]Department of Biology, Ashk.C., Islamic Azad University, Ashkezar, Iran. [2]Medical Biotechnology Research Center, Ashk.C., Islamic Azad University, Ashkezar, Iran. [3]Department of Industrial Environmental and Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran. [4]Department of Chemical Engineering, Faculty of Engineering, Ardakan University, Ardakan, Iran. [5]Department of Biology, Yazd University, Yazd, Iran.

## Abstract

**Introduction:** Breast cancer is currently the leading cause of cancer-related mortality among women worldwide. If urgent actions are not taken, the number of women diagnosed with breast cancer globally is projected to nearly double. Treating cancer cells is a complex process due to the presence of diverse biological pathways. Therefore, the simultaneous delivery of multiple drugs can exert a synergistic effect on the treatment process. Additionally, nanocarriers must exhibit high biocompatibility with healthy cells. Advancements in nanocarrier-mediated drug delivery offer promising strategies for improving anticancer agent efficacy. **Objective:** This study synthesized two novel copper Schiff-base complexes N, N'-bis (3-ethoxysalicylidene)-2,2-dimethyl-1,3-propanediamine Cu (II) ($CuL^{3OEt}$) and N, N'-bis (5-bromosalicylidene)-2,2-dimethyl-1,3-propanediamine Cu (II) ($CuL^{5Br}$) and compared their cytotoxicity against MCF-7 breast cancer cells in free and niosome-encapsulated forms. **Materials and Methods:** Niosomes were prepared via the thin-film hydration method to enhance delivery efficiency. Characterization using Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS) revealed uniform nanoparticles (87.8–243.3 nm). **Results:** Cytotoxicity, assessed via MTT assay, showed niosome-encapsulated forms significantly reduced $IC_{50}$ values (e.g., $CuL^{3OEt}$: 8.24 µg/mL vs. 80.65 µg/mL free form at 72 hr, $p < 0.05$), indicating improved potency. Substituent effects (ethoxy vs. bromo) influenced outcomes, linked to hydrophobicity differences (logS -4.788 vs. -5.75). Given the higher molecular weight of $CuL^{5Br}$ (530 g/mol) compared $CuL^{3OEt}$ (460 g/mol), the loading efficiency was 13.26% and 90.43%, respectively. **Conclusion:** These findings highlight niosomal delivery's potential to enhance Cu Schiff-base complex efficacy, laying a foundation for further preclinical studies.

**Keywords:** Cancer therapy- Schiff-base complexes- Niosomes- Drug delivery- Cytotoxicity- Breast cancer- MCF-7

*Asian Pac J Cancer Biol*, **11 (3)**, 729-737

Submission Date: 03/27/2026 Acceptance Date: 05/19/2026

## Introduction

Breast cancer presents a major global health burden, comprising roughly 11.7% of all cancer cases, while still being the most lethal cancer worldwide for women [1]. Innovative therapeutic agent which can effectively target the malignant cells have become due to the heterogeneity of breast cancer and drug resistance emergence [2]. In this regard, metal-based coordination compounds have shown activity in new anticancer drug development, especially copper (Cu) Schiff-base complexes [3]. Differently with the classical Au complexes, which have the only potential anticancer activity through their specific localization and binding with the genomic DNA, these copper complexes exhibit various biological activities, including DNA intercalation, reactive oxygen species (ROS) generation and mitochondrial function disturbance [4].

Among these vesicular carriers non-ionic surfactant-

**Corresponding Author:**
Dr. Mahmood Dehghani Ashkezari
Department of Biology, Ashk.C., Islamic Azad University, Ashkezar, Iran and Medical Biotechnology Research Center, Ashk.C., Islamic Azad University, Ashkezar, Iran.
Email: mdashkezary@yahoo.com

based vesicular carriers, known as niosomes, are increasingly recognized as a viable alternative for liposomes [5]. Niosomes are appreciated for their biocompatibility, non-expensiveness, and accommodation of hydrophilic and hydrophobic compounds [6, 7]. Niosome encapsulation has been shown to increase drug stability, circulation time and cellular uptake, ultimately leading to greater therapeutic outcomes [7, 8]. Using niosomes, it may be possible to take advantage of the existing anticancer properties of these complexes while minimizing their shortcomings and providing an easier way to treat cancers [9].

Herein, we study two synthesized Cu Schiff-base complexes, namely N,N'-bis (3-ethoxysalicylidene)-2,2-dimethyl-1,3-propanediamine Cu (II) ($CuL^{3OEt}$) and N,N'-bis (5-bromosalicylidene)-2,2-dimethyl-1,3-propanediamine Cu (II) ($CuL^{5Br}$) [10]. These compounds were substituted with different substituent groups ethoxy and bromo, respectively in order to assess the influence of structure on biological activity. The Schiff-base ligands, obtained from the compression of salicylaldehyde derivatives with diamines, act as versatile scaffold for coordination to metals, with copper addition able to further promote redox activities, an important component of cytotoxicity [11]. Previous studies have shown that by changing the substituent groups this could lead to changes in lipophilicity, electronic properties, and interactions with cellular targets that allow modulation of the anticancer activity. Despite that, the performances of similar complexes in either free or nanocarrier-encapsulated forms have not yet been systematically compared in the context of niosomal delivery [12-14].

The aim of this research was to synthesize and characterize $CuL^{3OEt}$ and $CuL^{5Br}$, encapsulate them within niosomes, and evaluate their anticancer activity against MCF-7 cells in comparison to their free forms.

## Materials and Methods

*2.1. Synthesis of Schiff-base complexes*

To a solution of 1 mmol (0.200 g) of Cu $(CH_3COO)_2.H_2O$ in 50 mL of MeOH has been added a solution of either $H_2L^{3OEt}$ or $H_2L^{5Br}$ (1 mmol) dissolved in 5 mL of hot MeOH. The resulting solution was held at reflux for 3 hours to get the precipitates of desired products, which were then filtered out and washed separately, using equal parts of $H_2O$ and MeOH (Figure 1).

*2.2. Spectrophotometric Determination of λ max and Calibration Curve Construction*

The spectrophotometric method was employed to determine the maximum wavelength (λ max). Initially, a dilution of the copper Schiff base complexes $CuL^{3OEt}$ and $CuL^{5Br}$ was prepared in DMSO solvent, and their absorption spectra were recorded in the range of 200 to 700 nm using a spectrophotometer (Epoch, USA). The absorption spectra were plotted for all concentrations, and the wavelength at which the maximum absorbance was observed across all concentrations was identified as the maximum wavelength (λ max). To construct the standard curve, various concentrations of the copper Schiff base complexes were prepared in two solvents: methanol and phosphate-buffered saline (PBS). Their absorbance at the maximum wavelength was then measured, and based on these values, the standard curves were plotted. The corresponding linear equations for each solvent were also calculated.

*Niosomes Preparation*

The thin-film hydration method was employed for the synthesis of niosomes containing copper(II) Schiff base complexes (Table 1). This method was carried out in both organic and aqueous phases. The copper Schiff base complexes were individually dissolved in chloroform and methanol. Tween 60, cholesterol, and 1,2-distearoyl-sn-glycero-3-PE (DSPE_mPEG) were added in a 60:40 ratios with a 2% concentration. The rotary evaporator settings were adjusted to 150 rpm for both phases, with a temperature of 45 °C for the organic phase and 55°C for the aqueous phase. Thin-film hydration was achieved by the addition of phosphate-buffered saline (PBS). Following the aqueous phase, probe sonication was employed to reduce particle size. The samples were sonicated for 40 minutes while immersed in an ice-water bath.

*The loading efficiency of two copper Schiff base complexes into niosomes*

To evaluate the encapsulation efficiency of the samples, the encapsulated and free extracts were first removed. Free copper Schiff base complexes $CuL^{3OEt}$ and $CuL^{5Br}$ were removed by dialysis using dialysis bags at 4 °C. Subsequently, the prepared niosomes were diluted tenfold using methanol to disrupt the lipid bilayer and release the encapsulated copper Schiff base complexes. In the next step, the absorbance of the released complexes was measured using a spectrophotometer at the maximum absorption wavelengths specific to each complex. Finally, the entrapment efficiency (EE) of the entrapped compound within niosomes was calculated by dividing the difference between the total amount used (Wtotal) and the free amount presented in the aqueous phase of supernatant (Wfree) by the total amount used of the compound according to the following formulae:

Equation 1:

$$EE\ \% = (W_{total} - W_{free})/W_{total} \times 100$$

*Atomic Force Microscopy*

Atomic Force Microscopy (AFM) was used to

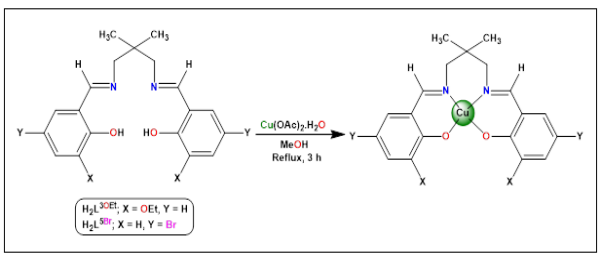


Figure 1. Syntheses of Copper (II) Complexes [$CuL^{3OEt}$ and $CuL^{5Br}$]

Table 1. The Synthesized Niosomal Formulations

| Formula | Tween 60 (%) | Cholesterol (%) | DSPE _mPEG (2000) (%) | Drug primary concentration | Total lipid to drug ration |
|---|---|---|---|---|---|
| $CuL^{5Br}$ | 60 | 40 | 2 | 400 µg/mL | 20 |
| $CuL^{3OEt}$ | 60 | 40 | 2 | 200 µg/mL | 20 |
| Blank | 60 | 40 | 2 | - | 20 |

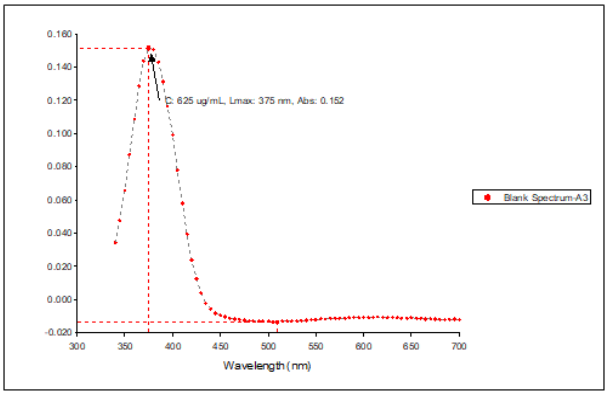


Figure 2. The UV-Vis Absorption Spectrum of $CuL^{5Br}$ was Recorded Using a Spectrophotometer to Determine Its Maximum Absorption Wavelength (λmax).

study the size and surface morphology of niosomes. A 10 µl of niosome suspension (1 mg/ml) was placed on freshly cleaved mica. The substrate was air dried at room temperature. Imaging was performed in tapping mode on a Bruker Dimension Icon AFM using a silicon cantilever (spring constant 40 N/m). 5 × 5 µm scan areas were analyzed and height profiles were processed using NanoScope Analysis software for determining dimensions and uniformity of particles.

*Dynamic Light Scattering*

The hydrodynamic diameter and polydispersity index (PDI) of niosomes were determined by Dynamic Light Scattering (DLS). DLS measurements were conducted on a Malvern Zetasizer Nano ZS at 25°C at a 173° scattering angle. To prevent multiple scattering effects, Niosome suspensions were diluted 1: 10 in distilled water. Results were expressed as mean diameter ± standard deviation, with each sample analyzed in triplicate.

*Zeta Potential*

The surface charge of niosomes was determined by Malvern Zetasizer Nano ZS. Samples were diluted 1:10 in distilled water and loaded into a folded capillary cell. Zeta potential measurement was performed at 25°C by electrophoretic light scattering technique and values were averaged over three runs. This parameter gave information about niosome stability and possible interactions with some membranes.

*Investigation of The Drug Release Profile from The Niosomal Delivery System*

To investigate the release of the complexes from the niosomal system over a period of 48 hours at 37 °C, the niosomes containing the copper (II) Schiff base complexes $CuL^{3OEt}$ and $CuL^{5Br}$ were placed inside dialysis bags and immersed in PBS solution. At predetermined time intervals, fixed volumes of the external medium were withdrawn and replaced with an equal volume of fresh, pre-warmed PBS. The absorbance of the collected samples was then measured using a UV-Vis spectrophotometer at the predetermined maximum absorption wavelength. The release profile of the complexes from the niosomes was plotted based on the standard calibration curve prepared in PBS.

*Cell Culture*

The human breast cancer cell line MCF-7 was purchased from National Cell Bank of Iran (NCBI), Pasteur Institute of Iran (NCBI, C131). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin (all from Gibco). The culture was maintained in a humidified incubator at 37°C with 5% $CO_2$. Cells were passaged every 2–3 days using 0.25% trypsin-EDTA (Gibco) upon reaching 80% confluence, and viability was confirmed

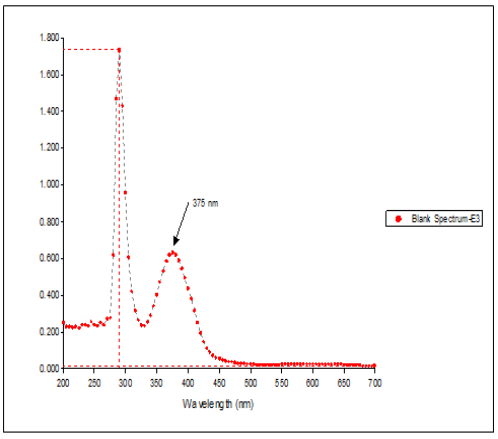


Figure 3. The UV-Vis Absorption Spectrum of $CuL^{3OEt}$ was Recorded Using a Spectrophotometer to Determine its Maximum Absorption Wavelength (λmax).

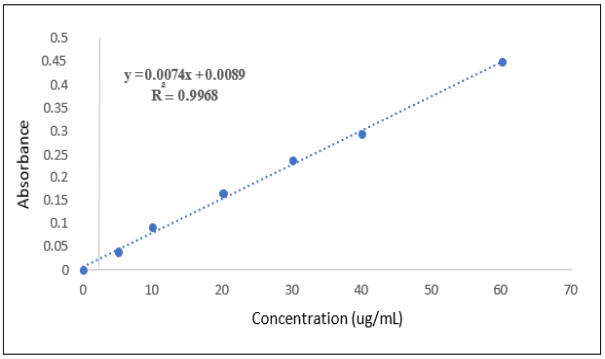


Figure 4. Calibration Curve of $CuL^{3OEt}$ in PBS

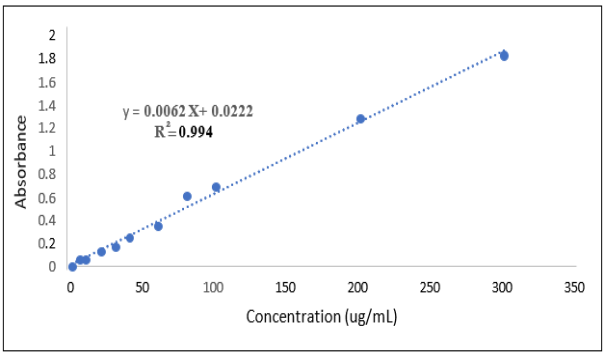


Figure 5. Calibration Curve of CuL$^{3OEt}$ in Methanol

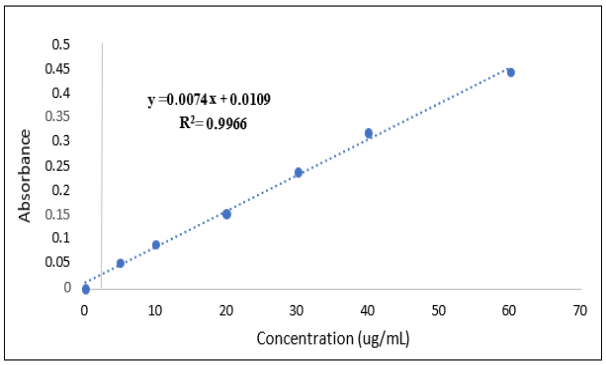


Figure 6. Calibration Curve of CuL$^{5Br}$ in PBS

via trypan blue exclusion prior to experiments (passages 5–15). MCF-7 cells were selected due to their widespread use as a model for estrogen receptor-positive breast cancer.

*Cytotoxicity Assay*

We investigated the cytotoxicity of free and niosome-encapsulated Cu Schiff-base complexes: (CuL$^{3OEt}$) and (CuL$^{5Br}$) in an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. To allow attachment, MCF-7 cells were plated in 96-well plates at the density of 1× 10$^4$ cells/well and incubated for 24 hr. Subsequently, cells were treated with serial dilutions (0.1–100 µg/ml) of free CuL$^{3OEt}$, free CuL$^{5Br}$, niosome-encapsulated CuL$^{3OEt}$, free niosome-encapsulated CuL$^{5Br}$ for 48 hr. Control cells included untreated cells and empty niosomes. After incubation, 20 µl of 5 mg/ml MTT solution in PBS (Sigma-Aldrich) was added into each well and the plates were incubated at 37°C for 4 hr. The generated formazan crystals were dissolved in 100 µl of dimethyl sulfoxide (DMSO) and the absorbance was measured with a microplate reader (BioTek ELx800) at 570 nm. Cell viability was expressed as a relative percentage of that determined in the controls, and IC$_{50}$ values were calculated by GraphPad Prism 10.0. All experiments were performed in triplicate.

*Statistical Analysis*

All values are expressed as mean ± standard deviation. Three independent experiments were performed. Data were analyzed using GraphPad Prism 10.0. (GraphPad, La Jolla, CA). One-way analysis of variance followed by Tukey's post hoc test. $p < 0.05$ was considered statistically significant.

## Results and Discussion

*Determination of Maximum Wavelength and Standard Curve*

The maximum wavelength for copper Schiff base complexes was determined using a spectrophotometer at various concentrations. The wavelength at which the copper Schiff base complexes CuL$^{3OEt}$ (Figure 2) and CuL$^{5Br}$ (Figure 3) showed the highest absorbance was 375 nm. Standard curves for copper Schiff base complexes CuL$^{3OEt}$ and CuL$^{5Br}$ were plotted in methanol and PBS solvents at their maximum absorption wavelength. Due to the direct relationship between sample concentration and absorbance, the curves were linear. For Schiff base copper complex CuL$^{3OEt}$ in PBS (Figure 4), the calibration curve was a straight line with an equation of Y=0.0074X+0.0089 and a coefficient of determination ($R^2$) of 0.9968 and CuL$^{3OEt}$ in methanol (Figure 5), the corresponding linear equation was Y=0. 0062X+0.0222 with an $R^2$ of 0.994. Similarly, for Schiff base copper complex CuL$^{5Br}$, the standard curve in PBS (Figure 6) had a linear equation of Y=0. 0074X+0. 0109 with an $R^2$ of 0.9966, and in methanol (Figure 7), the equation was Y=0. 0057X+0. 0021with an $R^2$ of 0.9983.

*Encapsulation efficiency of the niosomal system and evaluation of the drug release profile*

The encapsulation efficiency of the complexes within the niosomal system was calculated based on the

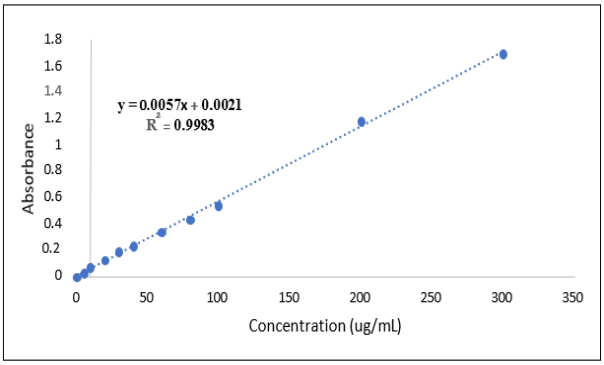


Figure 7. Calibration Curve of CuL$^{5Br}$ in Methanol

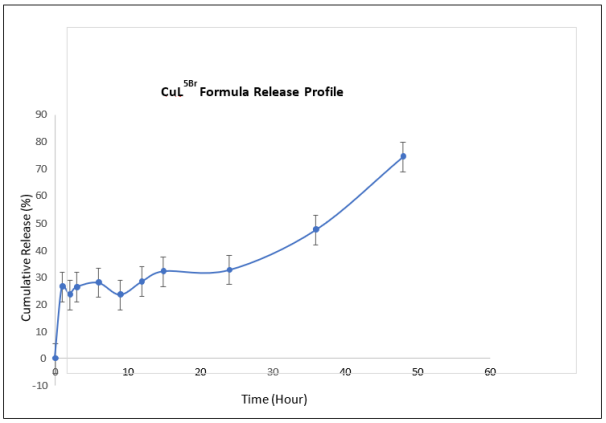


Figure 8. Release Profile of CuL$^{5Br}$ from the Niosomal Delivery System

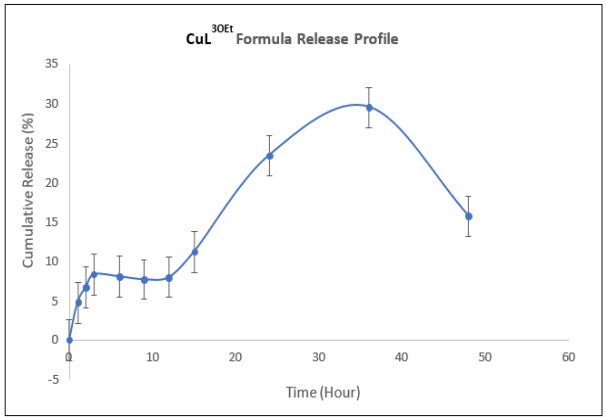


Figure 9. Release Profile of $CuL^{3OEt}$ from the Niosomal Delivery System

standard curve of the complex in methanol and Equation 1. The encapsulation efficiency values for samples $CuL^{3OEt}$ and $CuL^{5Br}$ were determined to be 90.43% and 13.26%, respectively. Analysis of the drug release profile revealed that although the $CuL^{5Br}$ formulation had a very low loading efficiency, it achieved nearly 80% release by the final hour (Figure 8). In contrast, formulation $CuL^{3OEt}$ (Figure 9) did not exhibit such a release pattern; its maximum release reached only 30%, followed by a subsequent decline.

Various literature sources indicate that both EE and drug loading capacity are affected by several variables, including the type of surfactant employed, drug solubility, and production techniques [25, 26]. Additionally, the molecular weight of an entrapped drug impacts EE, with lower molecular weight drugs resulting in higher EE due to their enhanced ability to integrate into the vesicle structure [27]. Investigations into the loading efficiency of copper Schiff base complexes within a niosomal system revealed that the $CuL^{5Br}$ complex (530 gr/mol), which has a higher molecular weight compared to the $CuL^{3OEt}$ complex (460 gr/mol), exhibited significantly lower loading capacity. This difference in molecular weight also influenced their solubility and release profiles from the niosomal system, with the higher molecular weight

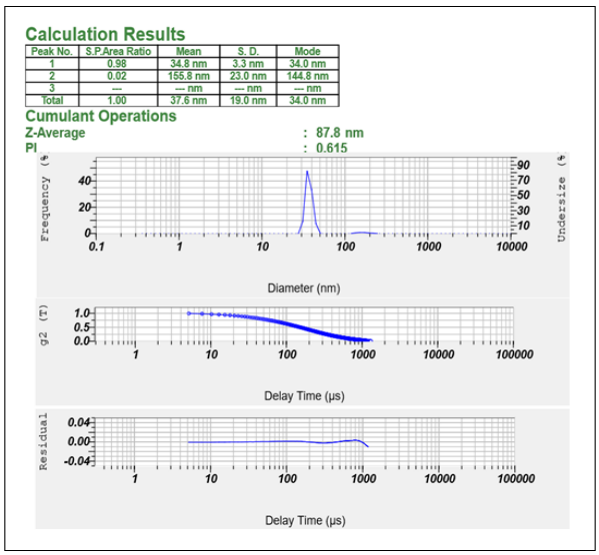


Figure 10. Blank Noisome DLS Analysis

$CuL^{5Br}$ complex demonstrating reduced solubility and slower release compared to the $CuL^{3OEt}$ complex.

The characterization of niosomal formulations via DLS revealed distinct size profiles and uniformity metrics. The Blank niosome exhibited an average hydrodynamic diameter of 87.8 nm with a polydispersity index (PDI) of 0.615, suggesting a relatively uniform particle size distribution (Figure 10). In contrast, niosomes loaded with $CuL^{3OEt}$ copper Schiff base complex displayed a larger mean size of 243.3 nm and a PDI of 0.454 (Figure 11), while those containing $CuL^{5Br}$ copper Schiff base complex measured 183.6 nm with a PDI of 0.354 (Figure 12). These measurements, consistent across triplicate analyses, indicate successful incorporation of the copper complexes into the niosomal structures, with the reduced PDI values in loaded formulations reflect improved homogeneity compared to the Blank niosome. The differential size between $CuL^{3OEt}$ and $CuL^{5Br}$ may be attributed to their structural variations ethoxy versus bromo substituents altering hydrophobicity and bilayer interactions. The logS values (-4.788 for $CuL^{3OEt}$, -5.75 for $CuL^{5Br}$), calculated via ChemDraw, indicate greater lipophilicity for $CuL^{5Br}$, potentially leading to tighter packing and smaller niosome size (183.6 nm vs. 243.3 nm), as observed in hydrophobic drug-loaded nanoparticles. In the study by Soni and colleagues [15], it was found that the characteristics of the materials and process variables affect vesicle size and entrapment efficiency.

Zeta potential analysis provided insights into the surface charge properties of the niosomes. The Blank niosome exhibited a strongly negative zeta potential of -63.4 mV, indicative of high colloidal stability due to electrostatic repulsion (Figure 13). Niosomes containing $CuL^{3OEt}$ and $CuL^{5Br}$ copper Schiff base complexes showed reduced negativity, with values of -16.2 mV (Figure 14)

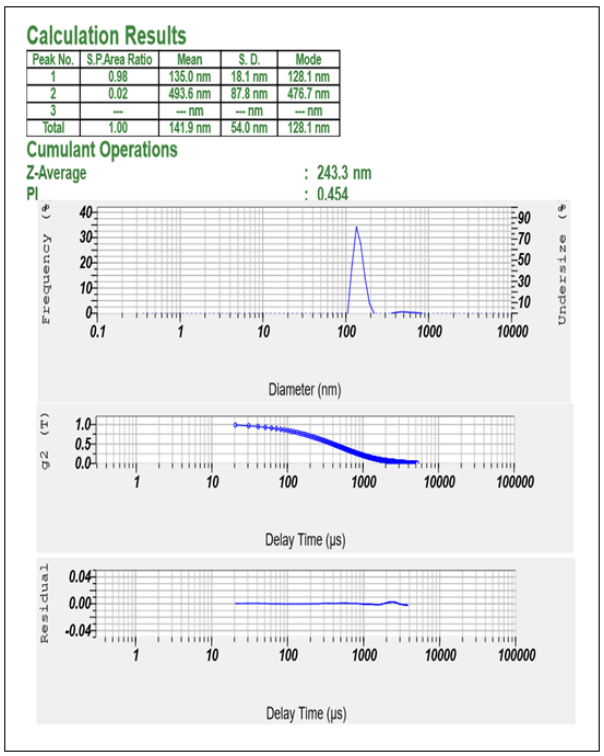


Figure 11. Niosomes Loaded with $CuL^{3OEt}$ DLS Analysis

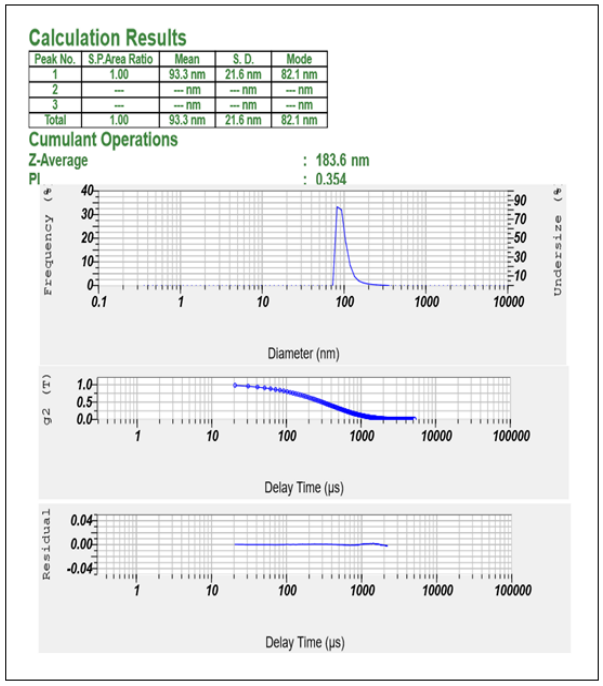


Figure 12. Niosomes Loaded with $CuL^{5Br}$ DLS Analysis

and -16.1 (Figure 15) mV, respectively, yet retained an anionic nature sufficient for stability. This shift likely reflects the surface modification induced by complex loading [16]. This finding is aligns with the results of the research by Kolahalam et al. [17], who suggested that a high zeta potential of more than 30 mV indicates superior colloidal physical stability, which is likely due to electrostatic repulsion between particles, and a decrease in zeta potential indicates potential particle aggregation.

AFM further confirmed the morphological integrity of these nanocarriers, revealing spherical, smooth-surfaced particles across all formulations Blank and loaded demonstrating homogeneity and structural consistency essential for biological applications (Figure 16). A similar result was obtained in the Ahmadi et al. [18] study. In this study, which investigated the effect of letrozole-loaded niosomes on breast cancer cells, the AFM results revealed that the niosomes exhibited a spherical shape.

Cytotoxicity assessments against MCF-7 breast cancer cells highlighted the differential effects of free versus niosomal formulations. The free $CuL^{3OEt}$ copper

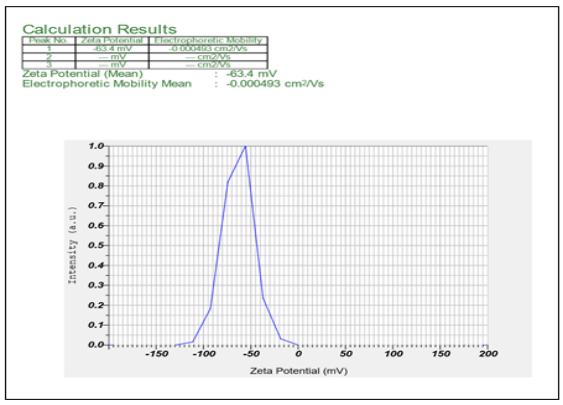


Figure 13. Determination of the Surface Charge of the Blank Niosomal System (mV)

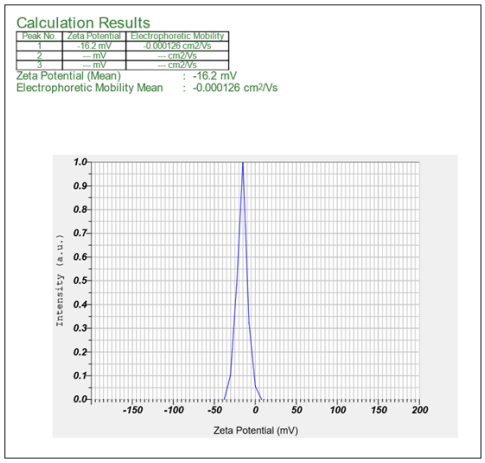


Figure 14. Determination of the Surface Charge of the $CuL^{3OEt}$ Niosomal System (mV)

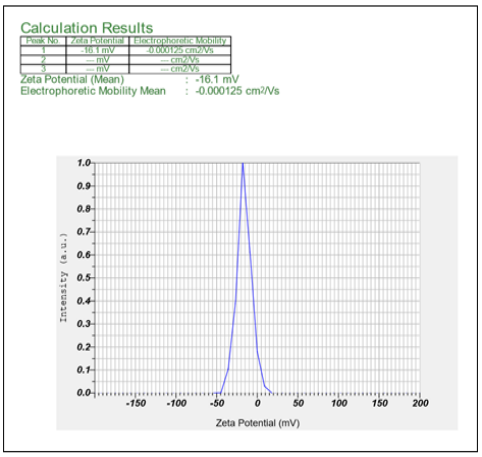


Figure 15. Determination of the Surface Charge of the $CuL^{5Br}$ Niosomal System (mV)

Schiff base complex yielded $IC_{50}$ values of 104.9 µg/mL, 95.91 µg/mL, and 90.34 µg/mL at 24, 48, and 72 hours, respectively, with significant cytotoxicity ($p < 0.05$) at higher doses (250 and 500 µg/mL). As shown in Figure 17, the free $CuL^{5Br}$ complex showed a time-dependent increase in potency, with $IC_{50}$ values decreasing from 193.5 µg/mL at 24 hours to 108.8 µg/mL at 48 hours and 47.29 µg/mL at 72 hours ($p < 0.05$).

In contrast, niosomal encapsulation markedly enhanced cytotoxicity. The $CuL^{3OEt}$-loaded niosome achieved $IC_{50}$ values of 10.78 µg/mL, 4.87 µg/mL, and 4.86 µg/mL over the same time points, with maximal efficacy at 90.43 µg/mL after 72 hours. The $CuL^{5Br}$-loaded niosome recorded $IC_{50}$ values of 51.35 µg/mL, 23.325 µg/mL, and 23.14 µg/mL, with peak cytotoxicity at 26.5 µg/mL (Figure 18). These results align with studies on niosomal delivery of metal complexes, such as Cu (II)-curcumin niosomes against MCF-7 cells ($IC_{50}$ reduction from 35 µg/mL to 12 µg/mL), where encapsulation improved bioavailability [19]. Similarly, niosomal doxorubicin reduced $IC_{50}$ by 5-fold in MCF-7 cells compared to its free form, underscoring the efficacy of vesicular carriers [20].

Comparative analysis underscored the superiority of niosomal delivery. The $CuL^{3OEt}$ niosomal formulation exhibited a 10- to 20-fold reduction in $IC_{50}$ compared to

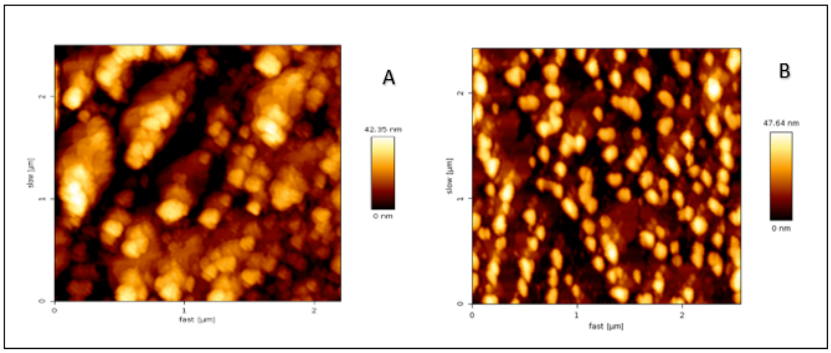


Figure 16. The $CuL^{3OEt}$ (A) and the $CuL^{5Br}$ (B) Niosomal Systems

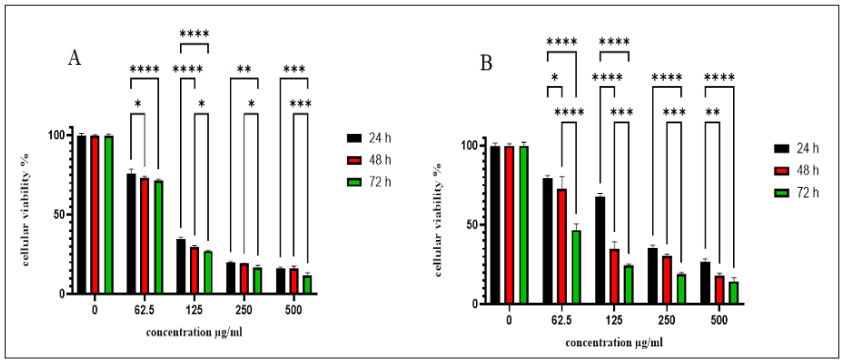


Figure 17. The Cytotoxic Effect of the Free $CuL^{3OEt}$ (A) and the Free $CuL^{5Br}$ (B) Complexes at Concentrations (62.5-500 µg/ml) on MCF-7 Cells. *, **, *** and **** shows significant at ≤ 0.05, ≤0.01, ≤0.001 and ≤0.0001, respectively.

its free form, while the $CuL^{5Br}$ niosomal system showed a 2- to 4-fold enhancement, particularly at 48 and 72 hours, where results stabilized. These findings suggest that niosomal encapsulation enhances cellular uptake and bioavailability, consistent with nanoparticle-mediated delivery mechanisms. The consistent efficacy at later time points highlights the potential of these niosomes as sustained-release anticancer agents. In the present study, the cytotoxicity of empty niosomes was evaluated in MCF-7 cells to determine their non-toxic concentration range. The results demonstrated that empty niosomes induced significant cell death only at concentrations higher than 500 µg/mg. In contrast, niosomes loaded with Schiff base compounds caused marked cytotoxic effects at substantially lower concentrations (Figure 18). These findings confirm that the observed cytotoxicity of the Schiff base–loaded niosomes is attributable to the encapsulated Schiff base compounds rather than to any inherent toxicity of the niosomal carrier itself.

In conclusion, this study highlights the significant potential of niosome-encapsulated copper Schiff base complexes, $CuL^{3OEt}$ and $CuL^{5Br}$, as potent anticancer agents against MCF-7 breast cancer cells. Niosomal delivery markedly enhanced cytotoxicity, reducing $IC_{50}$ values for $CuL^{3OEt}$ by 10- to 20-fold (from 80.65 µM to 3.73 µM at 72 hours) and for $CuL^{5Br}$ by 2- to 4-fold (from 148.73 µM to 17.78 µM), underscoring the critical role of nanocarriers in improving drug efficacy. The higher loading efficiency of $CuL^{3OEt}$ (90.43%) compared to $CuL^{5Br}$ (13.26%), attributed to differences in hydrophobicity (logS -4.788 vs. -5.75), emphasizes the influence of substituent effects on niosomal performance. Stable niosomes, with zeta potentials ranging from -63.4 to -16.1 mV and uniform spherical morphology, further confirm their suitability for therapeutic applications. These findings suggest that niosomal delivery could enhance the clinical utility of

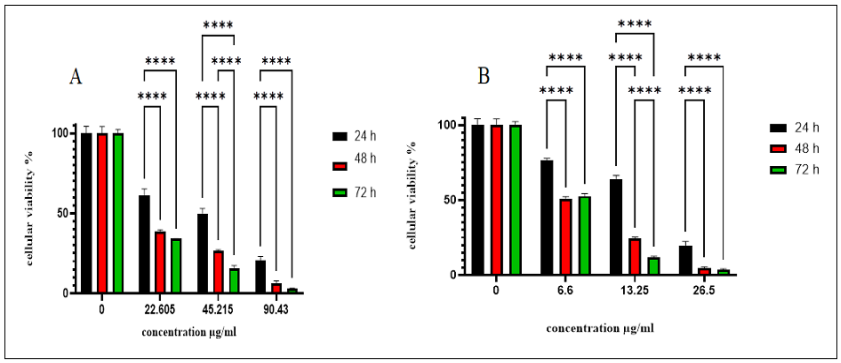


Figure 18. The Cytotoxic Effect of the $CuL^{3OEt}$- loaded Niosome (A) and the Free $CuL^{5Br}$- loaded Niosome (B) Complexes on MCF-7 Cells. *, **, *** and **** Shows Significant at ≤ 0.05, ≤0.01, ≤0.001 and ≤0.0001, Respectively.

metal-based complexes for breast cancer treatment. Future studies should explore underlying mechanisms, such as apoptosis or oxidative stress pathways, and evaluate in vivo efficacy to advance these complexes toward clinical application.

## Acknowledgements

None declared.

*Conflict of interests*

The authors declared no competing interests.

*Declaration of Interest Statement*

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

1. Sung H, Ferlay J, Siegel R, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a cancer journal for clinicians. 2021 05;71(3):209-249. https://doi.org/10.3322/caac.21660
2. Baliu-Piqué M, Pandiella A, Ocana A. Breast Cancer Heterogeneity and Response to Novel Therapeutics. Cancers. 2020 Nov 05;12(11):3271. https://doi.org/10.3390/cancers12113271
3. Karati D, Mukherjee S, Roy S. An Explicative Review on the Current Advancement in Schiff Base-Metal Complexes as Anticancer Agents Evolved in the Past Decade: Medicinal Chemistry Aspects. Medicinal Chemistry (Shariqah (United Arab Emirates)). 2023;19(10):960-985. https://doi.org/10.2174/1573406419666230707105221
4. Mertens RT, Gukathasan S, Arojojoye AS, Olelewe C, Awuah SG. Next Generation Gold Drugs and Probes: Chemistry and Biomedical Applications. Chemical Reviews. 2023 05 24;123(10):6612-6667. https://doi.org/10.1021/acs.chemrev.2c00649
5. Ge X, Wei M, He S, Yuan W. Advances of Non-Ionic Surfactant Vesicles (Niosomes) and Their Application in Drug Delivery. Pharmaceutics. 2019 01 29;11(2):55. https://doi.org/10.3390/pharmaceutics11020055
6. Patel BA. Niosomes: A Promising Approach for Advanced Drug Delivery in Cancer Treatment. International Research Journal of Modernization in Engineering Technology and Science. 2024 06 19;. https://doi.org/10.56726/IRJMETS52610
7. Moghtaderi M, Sedaghatnia K, Bourbour M, Fatemizadeh M, Salehi Moghaddam Z, Hejabi F, Heidari F, Quazi S, Farasati Far B. Niosomes: a novel targeted drug delivery system for cancer. Medical Oncology. 2022 09 29;39(12):240. https://doi.org/10.1007/s12032-022-01836-3
8. Yazdian-Robati R, Ahmadi SM, Mavandadnejad F, Ebrahimnejad P, Amirkhanloo S, Shad A. Liposome- and niosome-based drug delivery for pancreatic cancer. Recent Advances in Nanocarriers for Pancreatic Cancer Therapy. 2024 01 01;:95-114. https://doi.org/10.1016/B978-0-443-19142-8.00014-0
9. Liga S, Paul C, Moacă E, Péter F. Niosomes: Composition, Formulation Techniques, and Recent Progress as Delivery Systems in Cancer Therapy. Pharmaceutics. 2024 02 04;16(2):223. https://doi.org/10.3390/pharmaceutics16020223
10. Sahraei A, Kargar H, Hakimi M, Tahir MN. Synthesis, characterization, crystal structures and biological activities of eight-coordinate zirconium(IV) Schiff base complexes. Transition Metal Chemistry. 2017 09;42(6):483-489. https://doi.org/10.1007/s11243-017-0152-x
11. Santini C, Pellei M, Gandin V, Porchia M, Tisato F, Marzano C. Advances in copper complexes as anticancer agents. Chemical Reviews. 2014 01 08;114(1):815-862. https://doi.org/10.1021/cr400135x
12. Asgari S, Pourjavadi A, Setayeshmehr M, Boisen A, Ajalloueian F. Encapsulation of Drug-Loaded Graphene Oxide-Based Nanocarrier into Electrospun Pullulan Nanofibers for Potential Local Chemotherapy of Breast Cancer. Macromolecular Chemistry and Physics. 2021;222(15):2100096. https://doi.org/10.1002/macp.202100096
13. Mohan A, Narayanan S, Balasubramanian G, Sethuraman S, Krishnan UM. Dual drug loaded nanoliposomal chemotherapy: A promising strategy for treatment of head and neck squamous cell carcinoma. European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik e.V. 2016 02;99:73-83. https://doi.org/10.1016/j.ejpb.2015.11.017
14. Panagiotaki KN, Sideratou Z, Vlahopoulos SA, Paravatou-Petsotas M, Zachariadis M, Khoury N, Zoumpourlis V, Tsiourvas D. A Triphenylphosphonium-Functionalized Mitochondriotropic Nanocarrier for Efficient Co-Delivery of Doxorubicin and Chloroquine and Enhanced Antineoplastic Activity. Pharmaceuticals. 2017 Nov 21;10(4):91. https://doi.org/10.3390/ph10040091
15. Soni S, Baghel K, Soni ML, Kashaw SK, Soni V. Size-dependent effects of niosomes on the penetration of methotrexate in skin layers. Future Journal of Pharmaceutical Sciences. 2024 03 27;10(1):48. https://doi.org/10.1186/s43094-024-00624-2
16. Bai T, Xiang B, Li M, Manica R, Liu Q. Mechanism of modification of bitumen zeta potential by sodium citrate: Interactions between bitumen metal content and ligands. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2022 Nov 20;653:130007. https://doi.org/10.1016/j.colsurfa.2022.130007
17. Kolahalam LA, Prasad KRS, Krishna PM, Supraja N, Shanmugan S. The exploration of bio-inspired copper oxide nanoparticles: synthesis, characterization and in-vitro biological investigations. Heliyon. 2022 06;8(6):e09726. https://doi.org/10.1016/j.heliyon.2022.e09726
18. Ahmadi S, Seraj M, Chiani M, Hosseini S, Bazzazan S, Akbarzadeh I, Saffar A, Mostafavi E. In vitro Development of Controlled-Release Nanoniosomes for Improved Delivery and Anticancer Activity of Letrozole for Breast Cancer Treatment. International Journal of Nanomedicine. 2022;17:6233-6255. https://doi.org/10.2147/IJN.S384085
19. Abolhassani Targhi A, Mousavi-Niri N, Jaberi Ansari F, Jafari Mansoorian H. The Antibacterial Effects of Curcumin-Silver Nanoparticle and Curcumin-Copper Nanoparticle Loaded Niosomes. Health and Development Journal. 2022 07 10;11(1):1-8. https://doi.org/10.34172/jhad.2022.92066
20. Saharkhiz S, Zarepour A, Zarrabi A. Empowering Cancer Therapy: Comparing PEGylated and Non-PEGylated Niosomes Loaded with Curcumin and Doxorubicin on MCF-7 Cell Line. Bioengineering. 2023 Oct 02;10(10):1159. https://doi.org/10.3390/bioengineering10101159